

# Endothelin-1 as a Mediator of Fibrogenesis in the Development of Bronchiolitis Obliterans Syndrome post Lung Transplant

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## Introduction

Endothelin-1 (ET-1) appears to be a key mediator of endothelial and smooth muscle proliferation in pulmonary arterial hypertension (PAH) and may also mediate fibro-proliferation in pulmonary fibrosis (PF). The pro-inflammatory/fibrogenic effect of ET-1 in PAH and PF is suggested to occur via specific receptors ET<sub>A</sub> and ET<sub>B</sub>. Thus, dual receptor blockade has been postulated as a potential therapy for idiopathic pulmonary fibrosis (IPF).

Lung transplant bronchiolitis obliterans syndrome (BOS) is the leading cause of late mortality in lung transplant recipients (LTRs), characterised by airway inflammation, airway wall fibrosis and airway luminal obliteration. In common with IPF, the cytokine TGF- $\beta$  is over-expressed, which in turn may lead to increased ET-1 expression. We postulate that ET-1 may be an important mediator in the pathogenesis of lung transplant BOS, and thus ET<sub>A/B</sub> receptor antagonism is a potential therapeutic strategy for prevention or treatment of BOS post transplant.

The objectives of this study were to examine the association of ET-1 with lung allograft inflammation, elevated TGF- $\beta$ , IL-8 and airway fibrosis in BOS patients and to compare the distribution of ET-1 and ET<sub>A</sub> and ET<sub>B</sub> receptors in BOS airways and lung tissues from explanted lungs with IPF or PAH.

A series of four experiments was designed. The results of these experiments are presented in the context of four questions below.

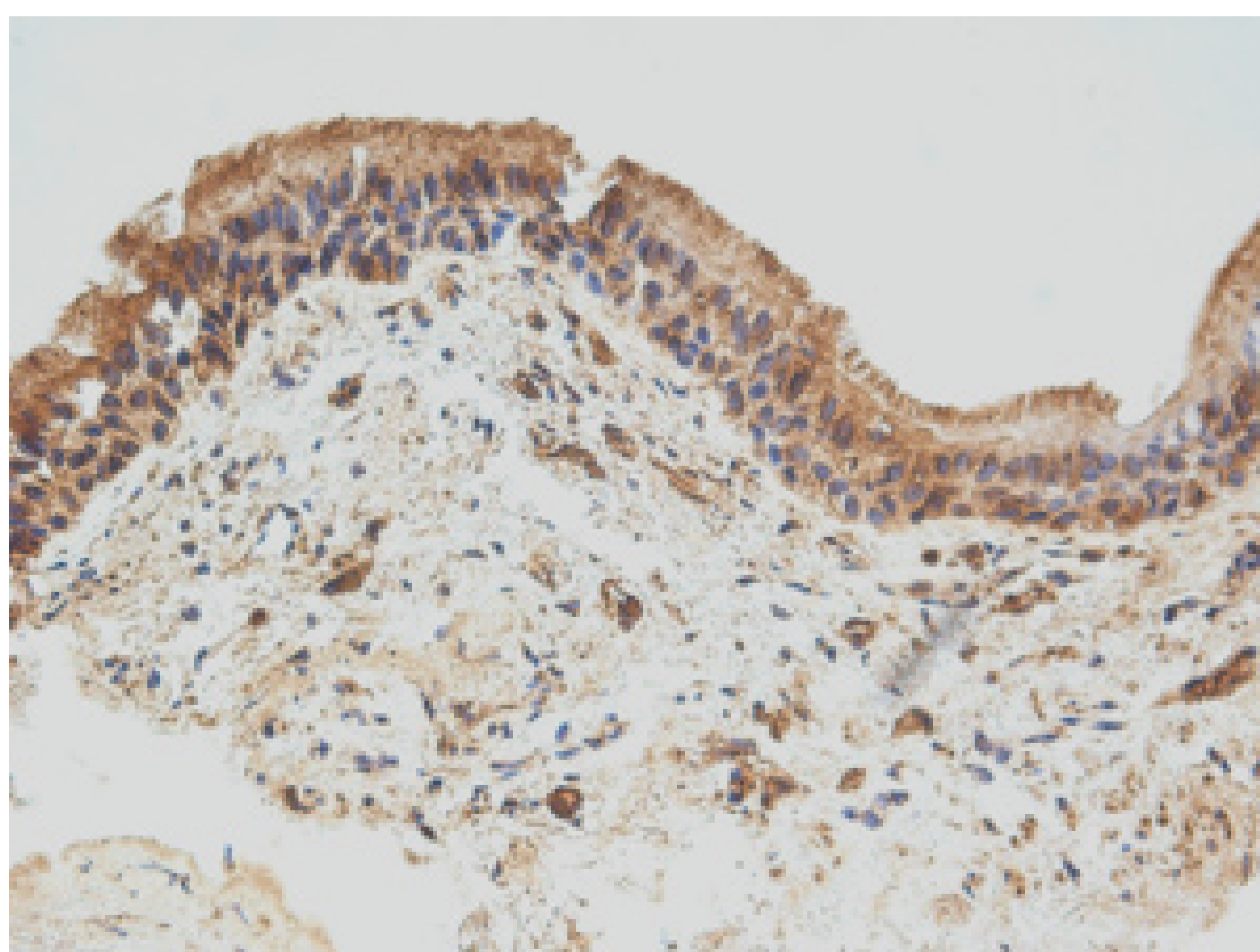
## Methods

- Fifty seven airway biopsies were taken from 22 initially stable LTRs over 3 years.
- ET-1 expression was evaluated by immunohistochemistry and quantified using a computer image analyser.
- BAL VEGF, TGF- $\beta$  and IL-8 levels were measured by a commercial ELISA kit.
- Bronchial epithelial cells (ECs) were taken by bronchial brush from 6 LTRs (5 clinically stable).
- ECs were co-cultured *in vitro* with or without patient's own peripheral blood mononuclear cells (PBMC) and ET-1 production by airway in the presence or absence of cyclosporin A (CsA) was measured by ELISA.
- Pulmonary vein lavage effluents were collected from 26 donors prior to transplantation.
- ET-1, IL-8, IL-10, IL-6 and VEGF levels in the retrograde flush effluent were measured from 42 lungs by ELISA using commercial kits.

## Results

### 1. Is ET-1 over-expressed as part of the inflammatory response resulting in the development of BOS post lung transplant?

Figure 1. Airway wall ET-1 expression in initially stable LTRs



ET-1 was mainly found in ECs, infiltrated cells and vessels (Figure 1).

Table 1. ET-1 is over-expressed prior to overt BOS post lung transplant

	Pre-BOS n = 22	Post-BOS n = 18	Never-BOS n = 4
ET-1 <sup>+</sup> airway wall cells	131 (78-246)*	218 (128-365)*	45 (26-85)
ET-1 <sup>+</sup> vessels	75 (32-106)*	43 (37-53)*	28 (6-37)

\* $p < 0.05$  compared with Never-BOS  
Results were expressed as positive cells or vessels per mm<sup>2</sup> of lamina propria.

In BOS group the airway ET-1<sup>+</sup> cells correlated with ET-1<sup>+</sup> vessels ( $r = 0.59$ ,  $p < 0.001$ ), BAL VEGF ( $r = 0.51$ ,  $p = 0.03$ ) and BAL IL-8 levels ( $r = 0.37$ ,  $p = 0.01$ ).

### 2. Is ET-1 expressed by bronchial ECs and can its expression be modulated by immunosuppressive agents?

Figure 2. ET-1 production by direct co-culture of bronchial ECs and PBMCs from LTRs

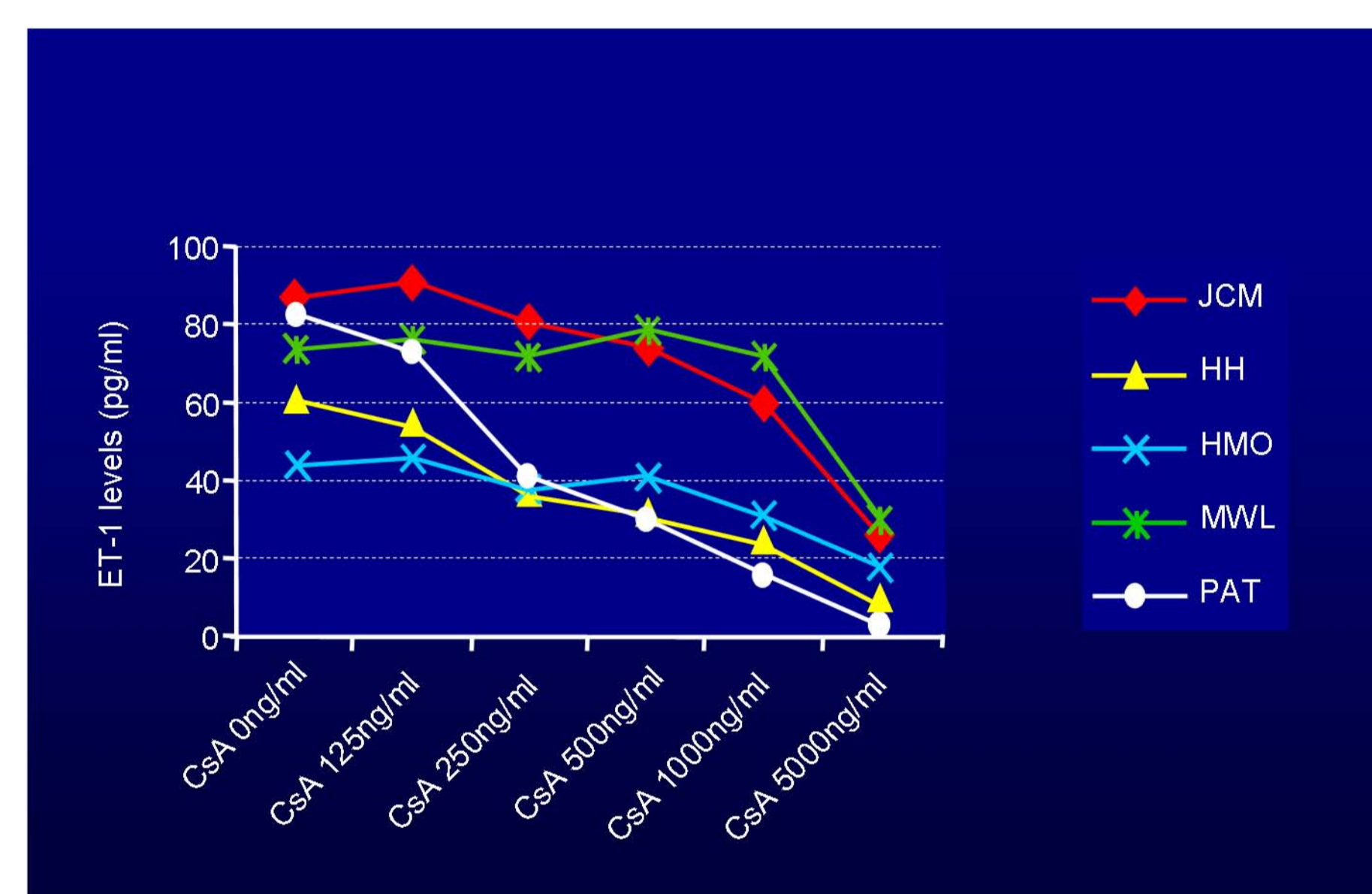
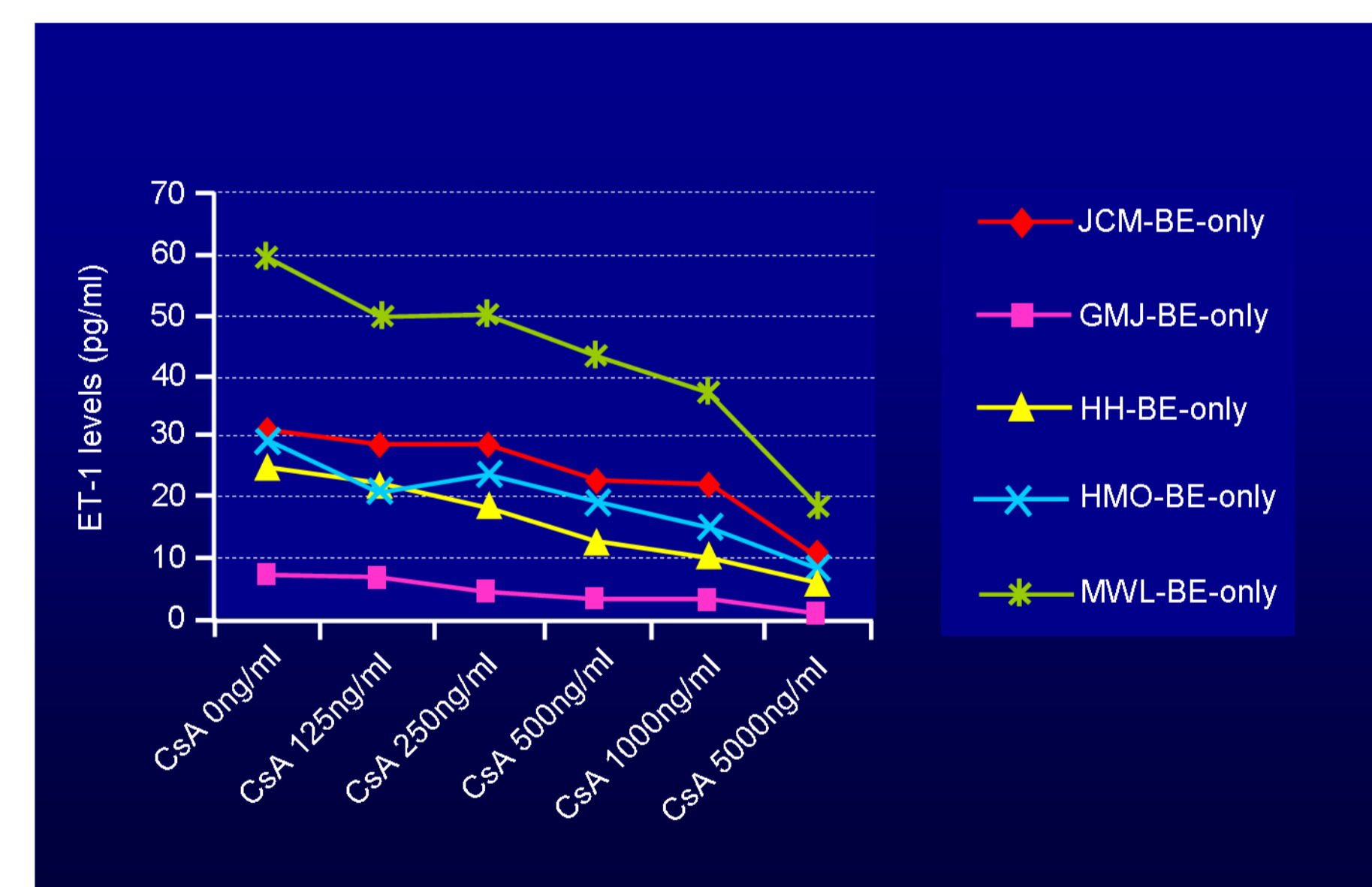


Figure 3. ET-1 production by bronchial ECs from LTRs

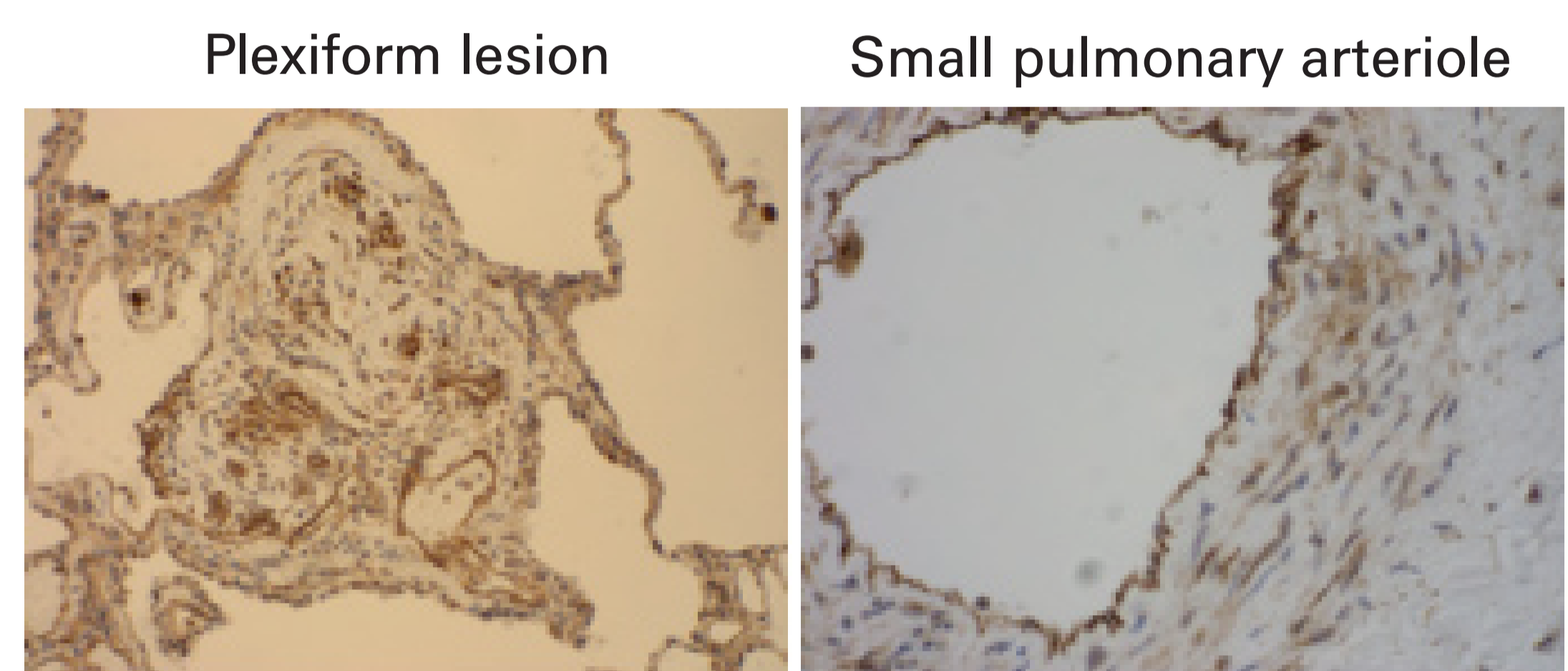


Epithelial cells were derived from 5 patients, whose initials are shown. Four patients are common to both and one is represented only in Figure 2 and one in Figure 3. BE = bronchial epithelial cells

ET-1 is expressed by bronchial ECs *in vitro* (Figures 2 and 3). CsA reduced ET-1 production in a dose-dependent manner. The suppression of ET-1 *in vitro* suggests that potential effects of CsA on the airway inflammatory response are not related to the direct immunological effects of CsA. PBMC culture alone did not produce detectable ET-1 levels.

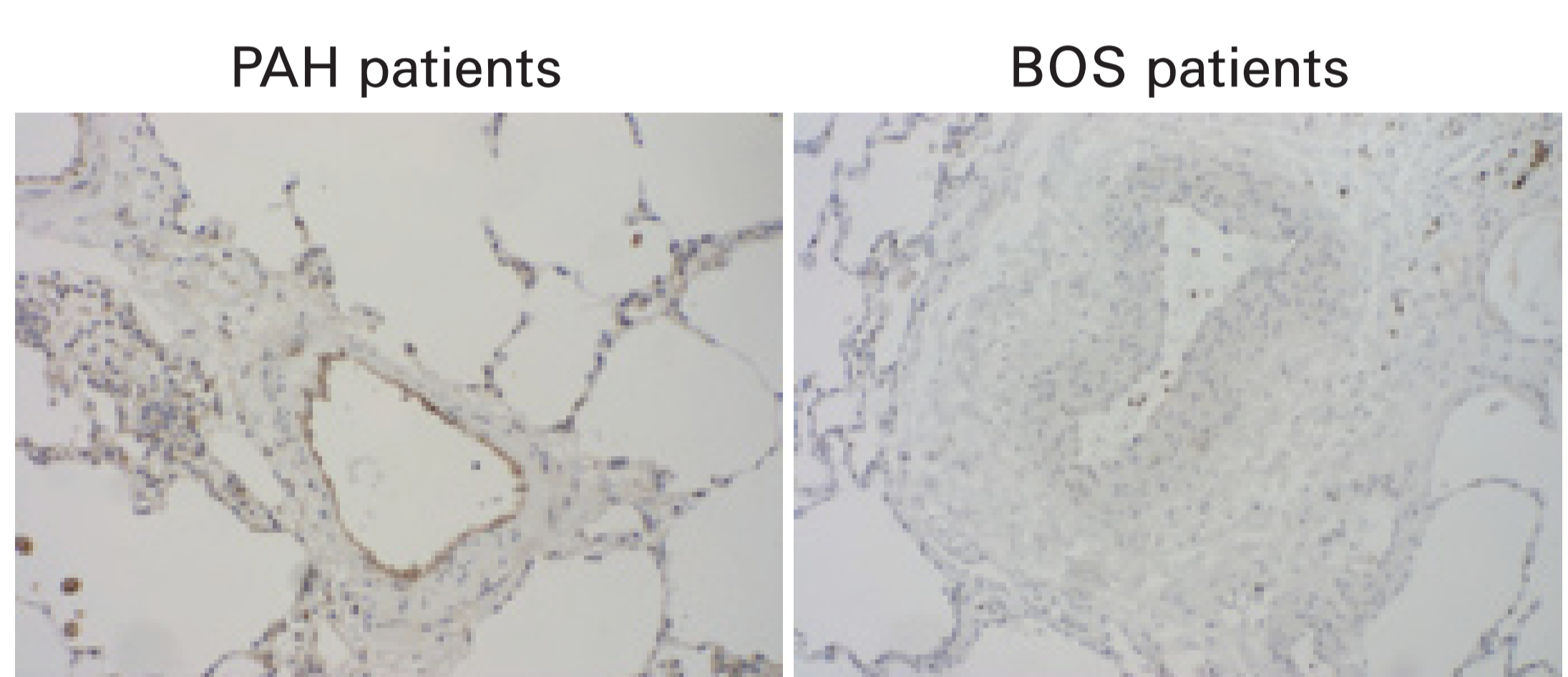
### 3. Does the histological location of ET-1 and ET<sub>A</sub> receptor expression vary with disease diagnosis and presence of pulmonary hypertension?

Figure 4. ET-1 staining in patients with PAH



ET-1 is expressed in plexiform lesions and small pulmonary arterioles of PAH patients (Figure 4).

Figure 5. ET<sub>A</sub> receptor staining in pulmonary arteriole



ET<sub>A</sub> receptor is observed in pulmonary arterioles of patients with PAH, but not in BOS patients (Figure 5). Over 40 explanted lung specimens (from the last 5 years) from patients with post lung transplant BOS, IPF, idiopathic PAH and Eisenmenger's PAH are currently being analysed.

### 4. Is ET-1 a marker of pre-transplant allograft injury?

Macroscopic pulmonary embolism (PE) was detected in 8 recipients (7 clot, 1 fat), the incidence of unexpected PE was 30.8%. The unexpected PE was significantly associated with increased IL-8 levels ( $p = 0.001$ ), but not with ET-1, IL-6 or IL-10.

## Summary and conclusions

- ET-1 is over-expressed even prior to overt BOS post lung transplant, suggesting a role in the pathogenesis of BOS.
- ET-1 is expressed by bronchial EC and its expression is reduced by CsA in a dose-dependent manner.
- ET<sub>A</sub> receptor is not expressed in pulmonary arterioles of BOS patients, in contrast to PAH patients.
- ET-1 does not appear to be a marker of pre-transplant allograft injury.
- ET-1 may mediate the development of BOS (perhaps stimulated by the presence of TGF- $\beta$ ). Although substantially more studies are required, ET receptor antagonism may represent a useful therapeutic strategy for post-transplant BOS.